I. AMENDMENTS

In the Title:

Please amend the title to read as follows:

E1 METHODS OF USING PROMISCUOUS G PROTEINS TO IDENTIFY G PROTEIN-COUPLED RECEPTORS AND THEIR LIGANDS.

In the Specification:

Please amend the first paragraph of the specification to read as follows:

E2 This application claims priority under 35 U.S.C. Section 119 to provisional patent application 60/020,234 filed on June 21, 1996, by Negulescu et al., which is herein incorporated by reference.

In the Claims

Please amend the following claims to read as follows:

63. (Twice amended) A method of identifying a G-protein coupled receptor (GPCR) for a given ligand, the method comprising:

E3 (i) providing a cell, said cell comprising,

a) a first heterologous inducible promoter operably linked to a first polynucleotide encoding a functional Gα15 protein having at least 95% sequence homology to SEQ. ID. NO. 2,

b) a second heterologous promoter operably linked to a second polynucleotide encoding a reporter gene, and

c) a third heterologous promoter operably linked to a third polynucleotide encoding said GPCR,

wherein said first heterologous inducible promoter provides for the low level expression prior to induction,

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wherein induction of said first heterologous inducible promoter provides for at least a three fold increase in expression of said Gα15 protein,

wherein induced expression of said Gα15 protein is sufficient to permit promiscuous coupling to said GPCR,

wherein said GPCR is not naturally expressed in said cell,

wherein said second heterologous promoter is directly or indirectly modulated by the activity of said Gα15 protein, and

wherein said cell arises from a cell line subjected to functional cell analysis with a signal transduction detection system;

(ii) contacting said cell with said ligand; and

(iii) detecting a change in reporter gene expression by comparing reporter gene expression prior to addition of said ligand with reporter gene expression after addition of said ligand.

71. (Three-times amended) A method for identifying a GPCR for a given ligand, the method comprising:

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(i) providing a cell, said cell comprising,

a) a first heterologous inducible promoter operably linked to a first polynucleotide encoding a functional Gα15 protein having at least 95% sequence homology to SEQ. ID. NO. 2, and

b) a second heterologous promoter operably linked to a second polynucleotide encoding said GPCR,

wherein said first heterologous inducible promoter provides for the low level expression prior to induction, and

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wherein induction of said first heterologous inducible promoter provides for at least a three fold increase in expression of said Gα15 protein, and

wherein induced expression of said Gα15 protein is sufficient to permit promiscuous coupling to said GPCR,

wherein said GPCR is normally coupled to either $G\alpha_i$, $G\alpha_s$ or $G\alpha_{12}$ in the absence of said Gα15 protein,

wherein said GPCR is not naturally expressed in said cell, and

wherein said cell arises from a cell line subjected to functional cell analysis with a signal transduction detection system;

(ii) contacting said cell with said ligand; and

(iii) detecting a change in a signal with a signal transduction detection system by comparing said signal prior to addition of said ligand with said signal after addition of said ligand,

wherein said signal transduction detection system comprises a dye.

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75. (Twice amended) A method of a identifying a ligand for a GPCR, the method comprising:

(i) contacting a cell with a test chemical, said cell comprising

a) a first heterologous inducible promoter operably linked to a first polynucleotide encoding a functional Gα15 protein having at least 95% sequence homology to SEQ. ID. NO. 2, and

b) a second heterologous promoter operably linked to a second polynucleotide encoding said GPCR,

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wherein said first heterologous inducible promoter provides for the low level expression prior to induction,

wherein induction of said first heterologous inducible promoter provides for at least a three fold increase in expression of said Gα15 protein,

wherein induced expression of said Gα15 protein is sufficient to permit promiscuous coupling to said GPCR,

wherein said GPCR is normally coupled to either $G\alpha_i$, $G\alpha_s$ or $G\alpha_{12}$ in the absence of said Gα15 protein,

wherein said GPCR is not naturally expressed in said cell, and

wherein said cell arises from a cell line subjected to functional cell analysis with a signal transduction detection system; and

(ii) detecting a change in a signal with a signal transduction detection system by comparing said signal prior to addition of said test chemical with said signal after addition of said test chemical,

wherein said signal transduction detection system comprises a dye,

wherein a change in reporter gene expression identifies the test compound as a ligand for the GPCR, thereby identifying the ligand for the GPCR.

81. (Three-times amended) A method of identifying a ligand for a GPCR, the method comprising

(i) contacting a cell with a test chemical, said cell comprising,

a) a first heterologous inducible promoter operably linked to a first polynucleotide encoding a functional Gα15 protein having at least 95% sequence homology to SEQ. ID. NO. 2,

b) a second heterologous promoter operably linked to a second polynucleotide encoding a reporter gene, and

c) a third heterologous promoter operably linked to a third polynucleotide encoding said GPCR,

wherein said first heterologous inducible promoter provides for the low level expression prior to induction, and

wherein induction of said first heterologous inducible promoter provides for at least a three fold increase in expression of said Gα15 protein, and

wherein induced expression of said Gα15 protein is sufficient to permit promiscuous coupling to said GPCR,

wherein said GPCR is not naturally expressed in said cell, and

wherein said second heterologous promoter is directly or indirectly modulated by the activity of said Gα15 protein, and

wherein said cell arises from a cell line subjected to functional cell analysis with a signal transduction detection system; and

(ii) detecting a change in reporter gene expression by comparing reporter gene expression prior to addition of said test chemical with reporter gene expression after

addition of said test chemical, wherein a change in reporter gene expression identifies the test compound as a ligand for the GPCR, thereby identifying the ligand for the GPCR.

88. (Amended) The method of claim 86, further comprising contacting said cell with phorbol myristate acetate or an analog thereof.

90. (Three-times amended) A method for identifying a modulator of signal transduction mediated by GPCR activation in a cell, the method comprising:

(i) contacting a cell with a ligand that in the absence of a test chemical, activates signal transduction in said cell, said cell comprising

a) a first heterologous inducible promoter operably linked to a first polynucleotide encoding a functional Gα15 protein having at least 95% sequence homology to SEQ. ID. NO. 2, and

b) a second heterologous promoter operably linked to a second polynucleotide encoding said GPCR,

wherein said first heterologous inducible promoter provides for the low level expression prior to induction, and

wherein induction of said first heterologous inducible promoter provides for at least a three fold increase in expression of said Gα15 protein, and

wherein induced expression of said Gα15 protein is sufficient to permit promiscuous coupling to said GPCR,

wherein said GPCR is normally coupled to either $G\alpha_i$, $G\alpha_s$ or $G\alpha_{12}$ in the absence of said Gα15 protein,

wherein said GPCR is not naturally expressed in said cell, and

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wherein said cell arises from a cell line subjected to functional cell analysis with a signal transduction detection system;

(ii) contacting said cell with the test compound, and

(iii) detecting a change in a signal with a signal transduction detection system by comparing said signal prior to addition of said test chemical with said signal after addition of said test chemical.

94. (Three-times amended) A method for identifying a modulator of signal transduction in a cell, the method comprising:

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(i) contacting a cell with a ligand that in the absence of a test chemical, activates signal transduction via a GPCR in said cell, said cell comprising,

a) a first heterologous inducible promoter operably linked to a first polynucleotide encoding a functional Gα15 protein having at least 95% sequence homology to SEQ. ID. NO. 2,

b) a second heterologous promoter operably linked to a second polynucleotide encoding a reporter gene, and

c) a third heterologous promoter operably linked to a third polynucleotide encoding said GPCR,

wherein said first heterologous inducible promoter provides for the low level expression prior to induction,

wherein induction of said first heterologous inducible promoter provides for at least a three fold increase in expression of said Gα15 protein,

wherein induced expression of said Gα15 protein is sufficient to permit promiscuous coupling to said GPCR,

wherein said GPCR is not naturally expressed in said cell,

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wherein said second heterologous promoter is directly or indirectly modulated by the activity of said Gα15 protein, and

wherein said cell arises from a cell line subjected to functional cell analysis with a signal transduction detection system;

(ii) contacting said cell with the test compound; and

(iii) detecting a change in reporter gene expression by comparing reporter gene expression prior to addition of said test chemical with reporter gene expression after addition of said test chemical.

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101. (Amended) The method of claim 99, further comprising contacting said cell with phorbol myristate acetate or an analog thereof.

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102. (Amended) A method of functionally profiling a test chemical comprising the steps of:

(i) contacting a panel of cells with a test chemical, said panel of cells comprising a plurality of cell clones, each cell clone comprising

a) a first heterologous inducible promoter operably linked to a first polynucleotide encoding a functional Gα15 protein having at least 95% sequence homology to SEQ. ID. NO. 2,

b) a second heterologous promoter operably linked to a second polynucleotide encoding a reporter gene, and

c) a third heterologous promoter operably linked to a third polynucleotide encoding said GPCR,

wherein said first heterologous inducible promoter provides for the low level expression prior to induction,

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wherein induction of said first heterologous inducible promoter provides for at least a three fold increase in expression of said Gα15 protein,

wherein induced expression of said Gα15 protein is sufficient to permit promiscuous coupling to said GPCR,

wherein said second heterologous promoter is directly or indirectly modulated by the activity of said Gα15 protein,

wherein said GPCR is not naturally expressed in said cell,

wherein said cell arises from a cell line subjected to functional cell analysis with a signal transduction detection system; and

wherein each cell clone differs only with respect to said GPCR that is expressed;

(ii) contacting said cell clones with a test chemical;

(iii) detecting reporter gene expression from said cell clones; and

(iv) comparing reporter gene expression between said cell clones.

E12 Amd 107. The method of claim 102, further comprising contacting said cells with a compound that increases calcium levels inside said cells.

E13 111. (Twice amended) The method of claim 110, wherein said reporter gene is β-lactamase.

E14 113. (Twice amended) The method of claim 112, wherein said reporter gene is β-lactamase.

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115. (Twice amended) The method of claim 114, wherein said reporter gene is β-lactamase.

116. (Twice amended) The method of claim 106, further comprising contacting said cell with a reporter gene substrate.

117. (Twice amended) The method of claim 106, wherein said reporter gene is β-lactamase.

118. (Twice amended) The method of claim 111, wherein said reporter gene substrate is CCF2.

119. (Twice amended) The method of claim 113, wherein said reporter gene substrate is CCF2.

120. (Twice amended) The method of claim 115, wherein said reporter gene substrate is CCF2.

121. (Twice amended) The method of claim 117, wherein said reporter gene substrate is CCF2.

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133. (Amended) The method of claim 63, wherein said method further comprises comparing said change in reporter gene expression detected in step (iii) with a change in reporter gene expression detected in a control cell line lacking said GPCR detected under the same conditions as in step (iii).

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134. (Amended) The method of claim 71, wherein said method further comprises comparing said change in signal detected in step (iii) with a change in signal detected in a control cell line lacking said GPCR detected under the same conditions as in step (iii).

135. (Amended) The method of claim 75, wherein said method further comprises comparing said change in signal detected in step (ii) with a change in signal detected in a control cell line lacking said GPCR detected under the same conditions as in step (ii).

136. (Amended) The method of claim 81, wherein said method further comprises comparing said change in reporter gene expression detected in step (ii) with a change in reporter gene expression detected in a control cell line lacking said GPCR detected under the same conditions as in step (ii).

137. (Amended) The method of claim 90, wherein said method further comprises comparing said change in reporter gene expression detected in step c) with a change in signal detected in a control cell line lacking said GPCR wherein said change is detected under the same conditions as in steps b) and c).

138. (Amended) The method of claim 94, wherein said method further comprises comparing said change in reporter gene expression detected in step (iii) with a change in reporter gene expression detected in a control cell line lacking said GPCR, detected under the same conditions as in step (iii).

Please add the following claims:

139. The method of claim 63, wherein the cell is a COS-7 cell comprising polynucleotides according to a, b, and c, wherein the first heterologous inducible promoter is a cytomegalovirus (CMV) promoter operably linked to a heptamerized tet operator, and wherein the cells further comprise a polynucleotide encoding a tetracycline-dependent transactivator operably linked to a constitutive promoter.

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140. The method of claim 71, wherein the cell is a COS-7 cell comprising polynucleotides according to a and b, wherein the first heterologous inducible promoter is a cytomegalovirus (CMV) promoter operably linked to a heptamerized tet operator, and wherein the cells further comprise a polynucleotide encoding a tetracycline-dependent transactivator operably linked to a constitutive promoter.

141. The method of claim 75, wherein the cell is a COS-7 cell comprising polynucleotides according to a and b, wherein the first heterologous inducible promoter is a cytomegalovirus (CMV) promoter operably linked to a heptamerized tet operator, and wherein the cells further comprise a polynucleotide encoding a tetracycline-dependent transactivator operably linked to a constitutive promoter.

142. The method of claim 81, wherein the cell is a COS-7 cell comprising polynucleotides according to a, b, and c, wherein the first heterologous inducible promoter is a cytomegalovirus (CMV) promoter operably linked to a heptamerized tet operator, and wherein the cells further comprise a polynucleotide encoding a tetracycline-dependent transactivator operably linked to a constitutive promoter.

143. The method of claim 90, wherein the cell is a COS-7 cell comprising polynucleotides according to a and b, wherein the first heterologous inducible promoter is a cytomegalovirus (CMV) promoter operably linked to a heptamerized tet operator, and wherein the cells further comprise a polynucleotide encoding a tetracycline-dependent transactivator operably linked to a constitutive promoter.

144. The method of claim 94, wherein the cell is a COS-7 cell comprising polynucleotides according to a, b, and c, wherein the first heterologous inducible promoter is a cytomegalovirus (CMV) promoter operably linked to a heptamerized tet operator, and wherein the cells further comprise a polynucleotide encoding a tetracycline-dependent transactivator operably linked to a constitutive promoter.

145. The method of claim 102, wherein the cell is a COS-7 cell comprising polynucleotides according to a, b, and c, wherein the first heterologous inducible promoter is a cytomegalovirus (CMV) promoter operably linked to a heptamerized tet operator, and wherein the cells further comprise a polynucleotide encoding a tetracycline-dependent transactivator operably linked to a constitutive promoter.--